

# AmpliTaq Gold® 360 Master Mix AmpliTaq Gold® 360 DNA Polymerase

360° Coverage for a Full Range of Targets



AmpliTaq® Gold 360 Master Mix and  
AmpliTaq® Gold 360 DNA Polymerase

## Benefits

- Optimized for the broadest range of targets (<3 Kb)—from everyday to challenging
- Unmatched sensitivity, specificity, and yield
- Robust amplification of GC-rich sequences with market-leading 360 GC Enhancer
- Achieves the highest quality sequencing data
- Available as an easy-to-use, premixed master mix

## Introduction

The new AmpliTaq Gold® 360 products provide you with a hot-start solution that is designed to amplify the entire 360 degree “circle” of target space—from everyday to more challenging templates. AmpliTaq Gold 360 products are available as a newly-formulated, superior master mix and a stand-alone enzyme with the improved AmpliTaq Gold 360 Buffer Kit which includes the new AmpliTaq Gold 360 Buffer, MgCl<sub>2</sub>, and a supplementary 360 GC Enhancer.

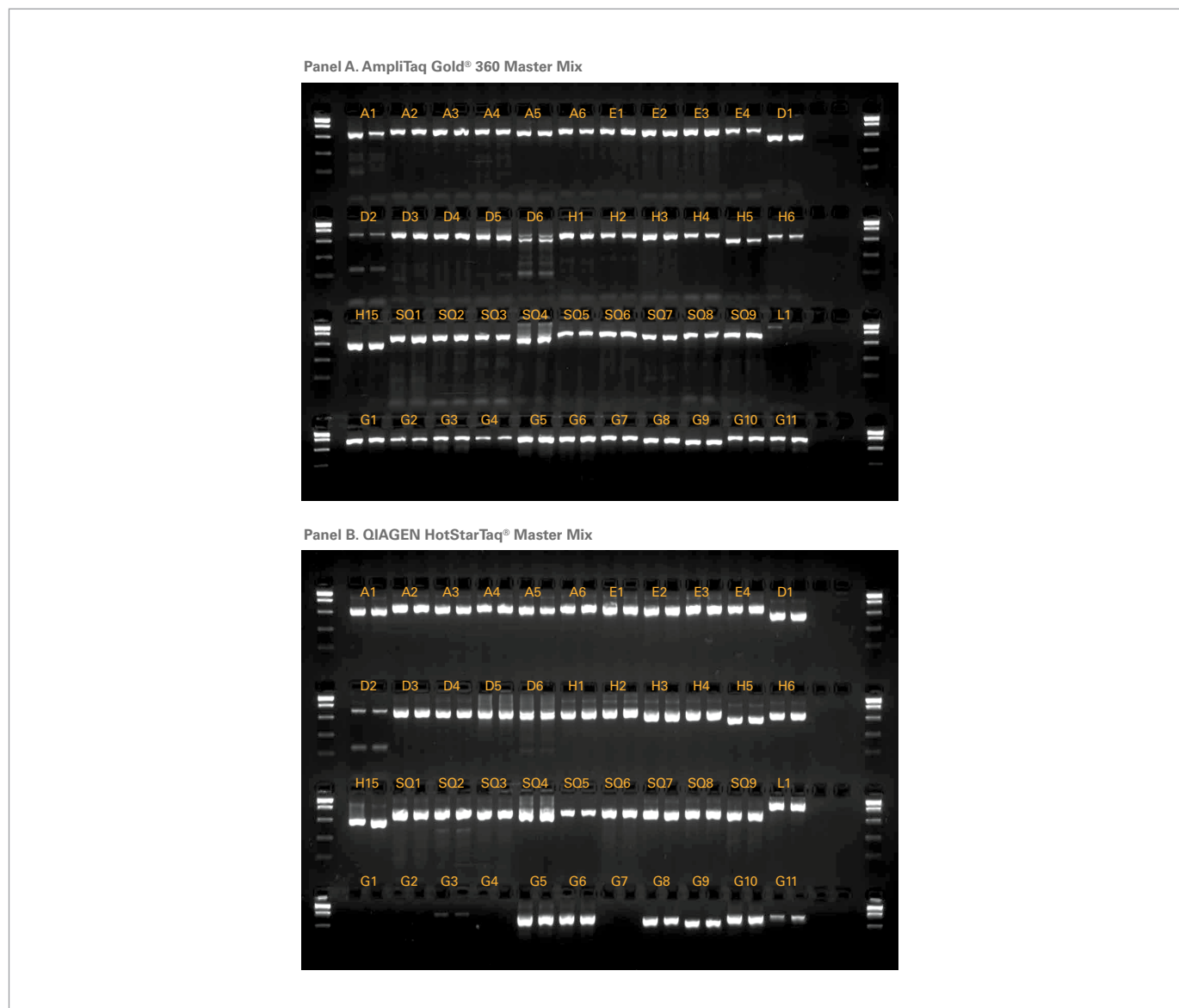
With or without the 360 GC Enhancer, the new AmpliTaq Gold 360 products have the best performance compared with other Taq DNA polymerases, including the original industry-leading AmpliTaq Gold DNA Polymerase—the most referenced brand of DNA polymerase in the world.

## Optimized for Easy and Challenging Targets

AmpliTaq Gold 360 DNA Polymerase, when used with the improved AmpliTaq Gold 360 Buffer and the optional 360 GC Enhancer, amplifies a vast range of DNA sequence contexts. AmpliTaq Gold 360 DNA Polymerase has been extensively tested and optimized for specificity and yield across a broad panel of both easy and difficult targets to achieve best-in-class performance for targets <3 Kb in length. Challenging targets include AT-rich, GC-rich, primer-dimer forming amplicons, homopolymer repeats, and amplicons that pose sequencing challenges. Targets that previously required significant optimization can now be reproducibly amplified with a single reagent under standardized conditions (Figure 1).

Description	AmpliTaq® Gold 360 DNA Polymerase	Roche FastStart Taq DNA Polymerase	Sigma JumpStart™ Taq Polymerase
	<b>Specific Yield (ng)</b>	<b>Specific Yield (ng)</b>	<b>Specific Yield (ng)</b>
Avg w/o GC-rich Amplicons	1196.65	936.41	1396.51
Avg GC-rich Amplicons	925.09	655.86	161.69
Avg all Amplicons	1110.24	847.15	1003.62
	<b>Specificity (%)</b>	<b>Specificity (%)</b>	<b>Specificity (%)</b>
Avg w/o GC-rich Amplicons	93.44	94.19	82.53
Avg GC-rich Amplicons	99.00	92.86	20.11
Avg all Amplicons	95.21	93.76	62.67

**Table 1. Average Specific Yield and Specificity for AmpliTaq Gold® 360 Taq DNA Polymerase Compared with Roche FastStart Taq Polymerase and Other Competitors.** PCR reactions were performed using 1 ng of template DNA per reaction and cycling conditions according to each manufacturer's recommendations. All enzyme concentrations were standardized at 0.025U/μL. Annealing and extension times and temperatures were specific to each primer set. Amplicons ranged from 300 to 1400 base pairs (bp) in length with an average length of 553 bp.

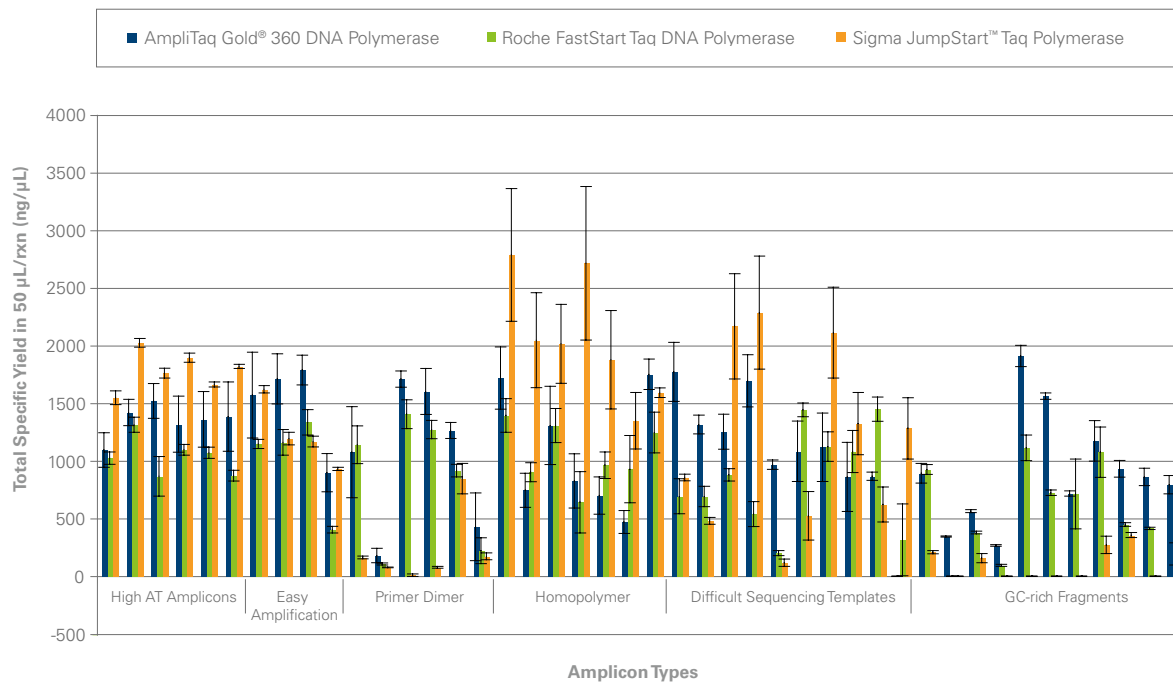


**Figure 1. AmpliTaq Gold® 360 Master Mix Amplifies a Broad Range of Targets.** Panel A shows products amplified with AmpliTaq® Gold 360 PCR Master Mix while Panel B shows the same products amplified using the QIAGEN HotStarTaq® Master Mix. PCR reactions were performed using 1 ng of template DNA per reaction using cycling conditions according to each manufacturer's recommendations. All enzyme concentrations were standardized at 0.025U/μL. Annealing temperatures were uniform across the selected targets. Amplicons ranged from 300 to 1400 base pairs (bp) in length with an average length of 553 bp. Each reaction was performed in duplicate. Amplicons are labeled as follows: E=Easy Amplification; A=High AT; G=High GC, L=Long; D=Primer Dimer; H=Homopolymer; SQ=Sequencing Challenge. The high GC amplicon (G) reactions included 5 μL of 360 GC Enhancer.

**Panel A. Specificity**



**Panel B. Specific Yield**



**Figure 2. AmpliTaq Gold® 360 DNA Polymerase Demonstrates Overall Superior Sensitivity and Specific Yield.** Summary of 2 replicates for each of 40 amplicons indicating the average specificity for each amplicon and the standard deviation. PCR reactions were performed using 1 ng of template DNA and 1.25 units of enzyme in each 50  $\mu$ L reaction. Annealing and extension times and temperatures were specific to each primer set. Amplicons ranged from 300 to 1400 base pairs (bp) in length with an average length of 553 bp. Specificity and specific yield averages are summarized in Table 1.

Benchmarking across more than 40 amplicons distinguishes AmpliTaq Gold® 360 DNA Polymerase as the best-performing enzyme, ensuring the highest probability of success for the amplification of both everyday and challenging targets (Table 1).

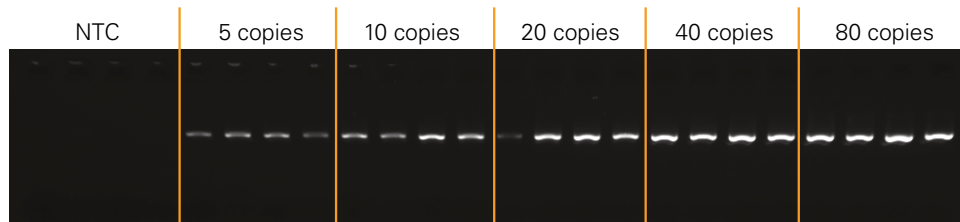
As shown in Figure 1, GC-rich regions are especially poorly amplified with other Taq DNA polymerases, while AmpliTaq Gold 360 DNA Polymerase provides successful, robust amplification.

### Superior Specificity and High Specific Yield

AmpliTaq Gold 360 DNA Polymerase provides the same hot-start specificity as AmpliTaq Gold DNA polymerase. A high-temperature incubation step is required to activate AmpliTaq Gold DNA Polymerase, which ensures that the active enzyme is generated only at temperatures in which the DNA is fully denatured and when the primers are not annealed.

When the polymerase is added to the reaction mixture at room temperature, primer extension does not occur because the enzyme is inactivated.

Any low stringency mispriming events that may have occurred will not be enzymatically extended and will not be amplified. Hence, PCR setup can be performed at room temperature without concern for extension at misprimed sites. The amount of AmpliTaq Gold 360 DNA Polymerase increases in the reaction slowly with each cycle number, and specific product yield increases without buildup of nonspecific products, including primer dimers. Excellent specificity across a broad range of targets (<3 Kb) is shown in Figure 1 and is summarized in Figure 2. This exquisite specificity allows easier multiplexing and allelic discrimination.



**Figure 3. Sensitivity of AmpliTaq Gold® 360 DNA Polymerase for Detection of Low Copy Targets.** Amplification of the  $\beta$ -actin gene was performed with 0 to 80 starting copies of DNA, using 2.0 mM  $MgCl_2$  and standard thermal cycling conditions with a 30 second extension time.

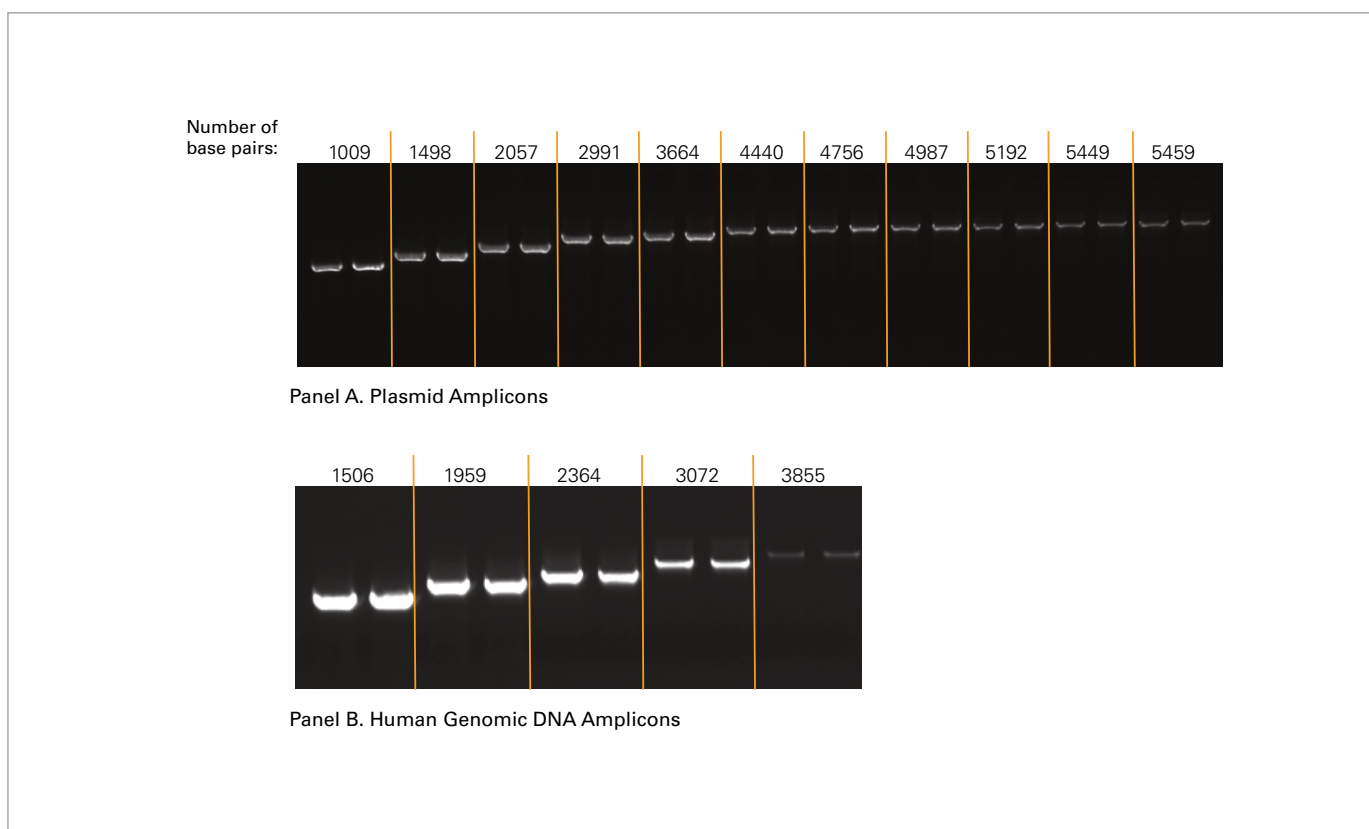
### Optimized 360 GC Enhancer

Applied Biosystems offers the best PCR buffer formulation combined with the top GC-rich enhancer solution for unsurpassed performance across the entire amplicon space. Each AmpliTaq Gold® 360 product includes the 360 GC Enhancer which is used for difficult templates and especially formulated for those sequences with a high percentage of GC content. The bottom row of amplicons in Figure 1 demonstrates the successful amplification of GC-rich sequences (designated as 'G') that other enzymes are unable to amplify. These data are summarized in Figure 2 to show that the average specificity of the high GC amplicons is higher for AmpliTaq Gold 360 DNA Polymerase than for other vendors.

### Superior Sensitivity and Amplification Length

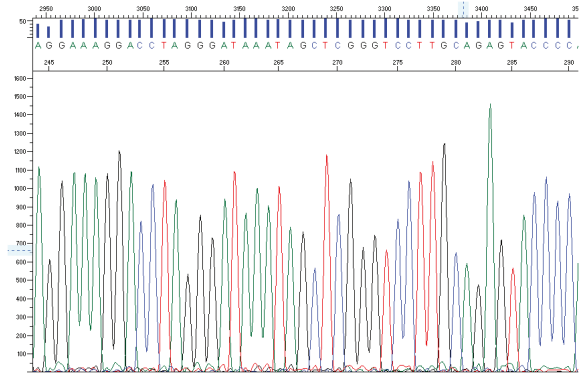
Compared to the original AmpliTaq Gold DNA Polymerase, AmpliTaq Gold 360 DNA Polymerase is purified by an additional proprietary separation process to eliminate contaminating bacterial DNA from the enzyme preparation. When used with the enhanced AmpliTaq Gold 360 Buffer, this ultra-pure enzyme, in addition to its hot-start capabilities, reduces false positive results, amplifies low-level target sequences, and promotes the amplification of a variety of templates including those from bacterial and human genomes.

AmpliTaq Gold 360 DNA Polymerase efficiently amplifies targets present at low copy number (Figure 3). Specific amplification occurs even in the presence of high concentrations of complex DNA, making it especially suited for low-copy pathogen detection, and amplification of targets from degraded DNA samples. The extreme purity of the enzyme contributes to its unmatched sensitivity. AmpliTaq Gold 360 DNA Polymerase efficiently and reproducibly amplifies long sequences (up to 3 Kb for hgDNA and 5 Kb for plasmid DNA). Figure 4 demonstrates robust PCR amplification of long human and plasmid DNA.

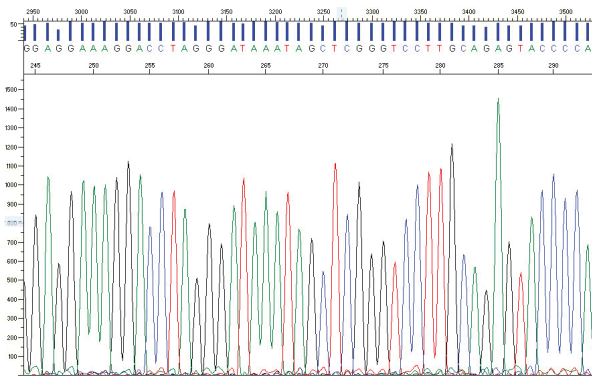


**Figure 4. Amplification of Long Targets with AmpliTaq Gold® 360 DNA Polymerase.** Both plasmid (Panel A) and human genomic DNA (Panel B) amplicons, up to 5459 bp long, were efficiently and reproducibly amplified in duplicate reactions. Plasmid DNA was amplified using 2 mM MgCl<sub>2</sub> and standard thermal cycling conditions with a 6 minute extension time. Human Raji cell DNA was amplified using 2 mM MgCl<sub>2</sub> and standard thermal cycling conditions with a 4 minute extension time.

**Panel A.**  
**Example of sequencing results from a standard template**



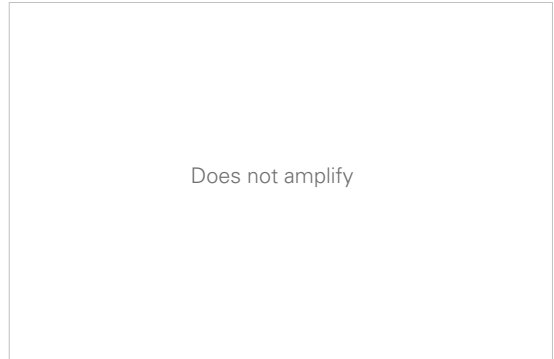
AmpliTaq Gold® DNA Polymerase



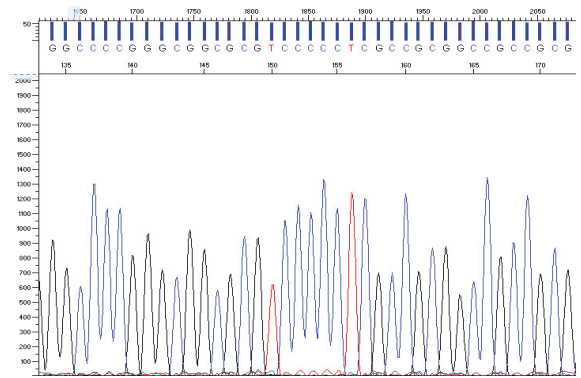
AmpliTaq Gold® 360 DNA Polymerase

	Length of Read (bp)	KB-QV30	Peak-under-Peak
AmpliTaq Gold® DNA Polymerase	553	538	0.0417
AmpliTaq Gold® 360 DNA Polymerase	547	536	0.0375
Amplicon Length: 634 bp GC Content: 57.4%			

**Panel B.**  
**Example of sequencing results from a high % GC template**



AmpliTaq Gold® DNA Polymerase



AmpliTaq Gold® 360 DNA Polymerase with 360 GC Enhancer

	Length of Read (bp)	KB-QV30	Peak-under-Peak
AmpliTaq Gold® DNA Polymerase	Does not amplify		
AmpliTaq Gold® 360 DNA Polymerase	532	522	0.0470
Amplicon Length: 573 bp GC Content: 79.9%			

**Figure 5. High Quality Sequencing Data Obtained from AmpliTaq Gold® 360 DNA Polymerase-Generated PCR Products.** Amplification products from both standard (Panel A) and high GC content (Panel B) targets generated by AmpliTaq Gold DNA Polymerase and AmpliTaq Gold DNA Polymerase were sequenced (10 ng PCR product per sequencing reaction). Samples were cycle-sequenced using BigDye® Terminator v3.1 and standard thermal cycling conditions. BigDye XTerminator™ Purification Kit was used to clean up the cycle sequencing reaction, and the samples were injected into the Applied Biosystems 3730xl DNA Analyzer with POP-7™ polymer.

### **Obtain High Quality Sequencing Data**

Achieve equivalent or better sequencing results compared to the original AmpliTaq Gold® DNA Polymerase. Quality parameters including read length, KB-QV30, and peak-under-peak were compared for sequencing data obtained from both AmpliTaq Gold 360 DNA Polymerase and the original AmpliTaq Gold DNA Polymerase (Figure 5).

### **Convenient, Easy-to-Use Hot-Start Master Mix**

AmpliTaq Gold 360 Master Mix contains everything required for successful PCR amplification in one convenient package, with all components premixed and premeasured. The master mix is supplied at 2X the recommended usage concentration for easy dilution when adding template and primers. Designed for convenience, the AmpliTaq Gold 360 Master Mix scales to various

reaction volumes for greater application and format flexibility. AmpliTaq Gold 360 Master Mix offers the best overall performance considering specific yield and convenience.

AmpliTaq Gold 360 DNA Polymerase is the key ingredient in an automated, convenient and efficient hot-start PCR. When AmpliTaq Gold 360 Master Mix is added to the reaction mixture at room temperature, the inactive enzyme is not capable of primer extension. Any low-stringency mispriming events that may have occurred will not be enzymatically extended and subsequently amplified. An initial thermal incubation step is required for activation and ensures that active enzyme is generated only at temperatures where the DNA is fully denatured.

### **Unparalleled PCR Technical Support**

Applied Biosystems has ongoing R&D programs that focus on refining the PCR process and developing new applications and complementary products. We offer technical seminars, PCR user-group meetings, and internet sites to keep you up-to-date on PCR protocols and new applications. In addition, our experienced staff of PCR specialists is always available to assist you.

For a list of national and international technical support phone numbers, visit our Web site at

**[www.appliedbiosystems.com](http://www.appliedbiosystems.com)**.

## ORDERING INFORMATION

Description	Size	P/N
AmpliTaq Gold® 360 Master Mix	5 mL	4398881
<i>Includes AmpliTaq Gold 360 Master Mix and 360 GC Enhancer</i>	50 mL	4398886
	10 x 5 mL	4398901
AmpliTaq Gold® 360 DNA Polymerase	250U	4398823
<i>Includes AmpliTaq Gold 360 DNA Polymerase, AmpliTaq Gold 360 Buffer, 25 mM MgCl<sub>2</sub> and 360 GC Enhancer</i>	1000U	4398833
	1500U	4398892
	2500U	4398843
	3000U (2 x 1500U)	4398894
	5000U (5 x 1000U)	4398896
	25 x 1000U	4398898
AmpliTaq Gold® 360 DNA Polymerase—standalone	2500U	4398900
AmpliTaq Gold® 360 Buffer Kit	1.5 mL	4398853
<i>Includes AmpliTaq Gold 360 Buffer, 25 mM MgCl<sub>2</sub> and 360 GC Enhancer</i>	6 x 1.5 mL	4398863
	150 mL	4398872
Protocol: AmpliTaq Gold® 360 Master Mix	1 protocol	4398944
Quick Reference Card: AmpliTaq Gold® 360 Master Mix	1 card	4398954
Protocol: AmpliTaq Gold® 360 DNA Polymerase	1 protocol	4398943
Quick Reference Card: AmpliTaq Gold® 360 DNA Polymerase	1 card	4398953

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Printed in the USA, 01/2009 Publication 104PB13-01



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